

# CLINICAL PROTEOMIC TUMOR ANALYSIS CONSORTIUM

*Interrogating Cancer Biology to Address  
Clinically Relevant Questions*

## **NCI-Clinical Proteomic Tumor Analysis Consortium: Building A Standardized Proteogenomic Pipeline for Understanding Cancer Biology**

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***Office of the Director***

***National Cancer Institute***

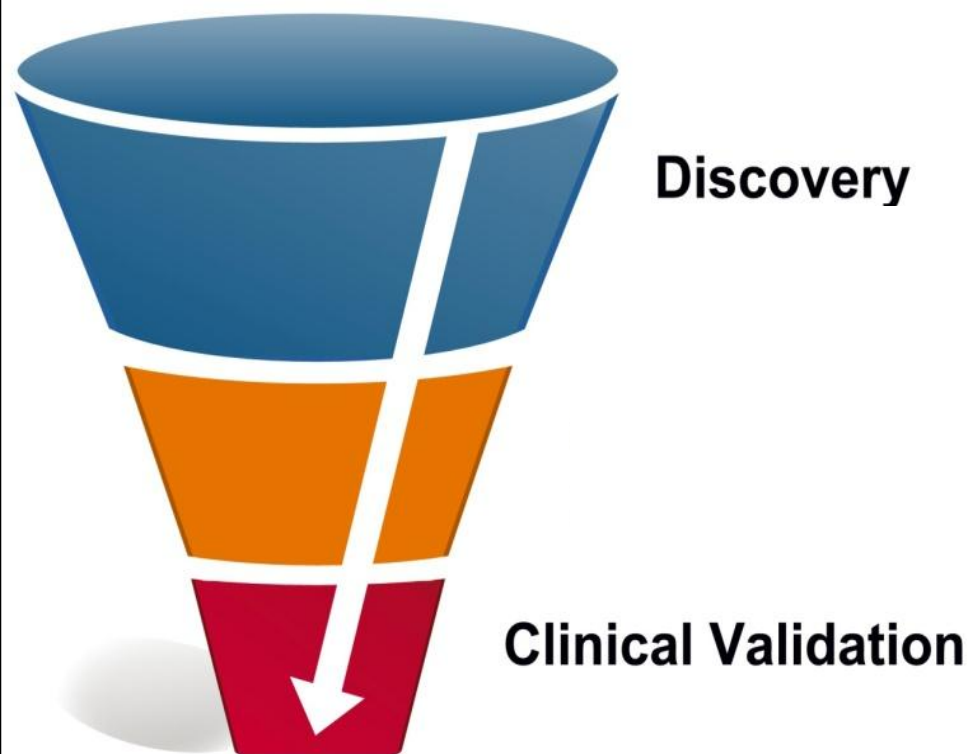


## *What is the background?*

- Analytical irreproducibility barriers using proteomics (false biomarker claims)
- No FDA-authorized MS-based proteomic assays at the time
- NCI-CPTAC launch in 2006 to address analytical issues
- NCI-TCGA large-scale genomics initiative in 2006 (extensively characterize large number of tumors)

# CPTAC's Multi-stage Standardized Proteomic Pipeline

*(Building proteomics competency to ensure reproducibility)*



## Round Robin Study Goal:

Demonstrate MRM assay accuracy and reproducibility across labs to measure proteins in plasma (low ug/ml with CV<22% at LOD)

- Platform Validation (TQMS) using SOPs and common material (research grade), open source software tool Skyline
- Immuno-MRM increases sensitivity by 1000-fold
- Community Adoption



Source: Addona, TA, et. al., Nat Biotechnol. **2009**, 27(7):633-641.

# Analytical Validation Workshop for Regulatory Device Clearance



- **Workshop (NCI-FDA IOTF MDx subcommittee):** analytical validation needs for multiplex proteomic technologies in clinical use

- **Outcome:** **Public** mock 510(k) pre-market applications
  - multiplex immuno-MRM assay (N. Leigh Anderson)
  - multiplex immunological array-based assay (Fred Regnier)

## Sources:

- Regnier, F, et. al. (2010) *Clinical Chemistry*. 56(2):165-171.
- Rodriguez, H, et. al. (2010) *Clinical Chemistry*. 56(2):237-243.
- Boja, E, et. al. (2011) *Clinical Chemistry*. 57(4):1-8.

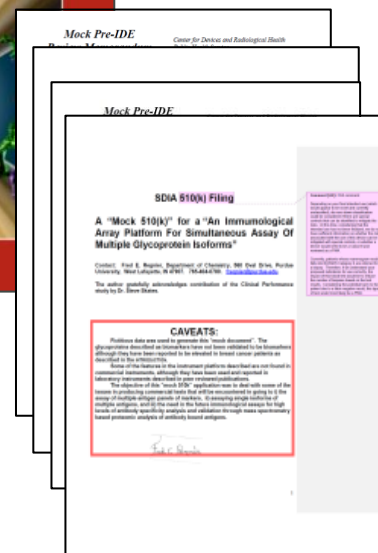
## Regulatory Science “cliffsnotes”

Special Issue



Reports

510(k) w/ FDA comments



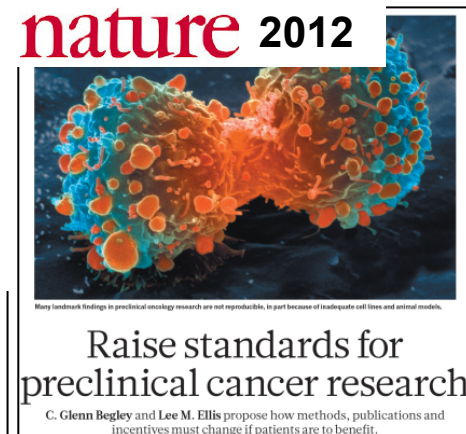


# Fit-for-Purpose Documents (Defining “What Is a Proteomics Targeted Assay”)



## What Is an “Assay” (targeted MS)?

- Workshop (2013) – establish information authors need to provide to enable reviewers & readers to understand what was done
- **Participants:** NCI, NHLBI, AACC, FDA, pharma, biotech, CRO, CMS, major journals
- **Outcome:** fit-for-purpose publication.
  - ***MCP journal expected to develop recommendations into pub guidelines***



### Targeted Peptide Measurements in Biology and Medicine: Best Practices for Mass Spectrometry-based Assay Development Using a Fit-for-Purpose Approach

Steven A. Carr<sup>1\*</sup>, Susan E. Abbatiello<sup>1</sup>, Bradley L. Ackermann<sup>2</sup>, <sup>3</sup>Christoph Borchers, <sup>4</sup>Bruno Domon, <sup>5</sup>Eric W. Deutsch, <sup>6</sup>Russell P. Grant; <sup>7</sup>Andrew N. Hoofnagle; <sup>8,9</sup>Ruth Hüttenhain; <sup>10</sup>John M. Koomen; <sup>11</sup>Daniel C. Liebler; <sup>12</sup>Tao Liu, <sup>7</sup>Brendan MacLean, <sup>1</sup>DR Mani, <sup>13</sup>Elizabeth Mansfield, <sup>14</sup>Hendrik Neubert, <sup>15</sup>Amanda G. Paulovich, <sup>16</sup>Lukas Reiter, <sup>17</sup>Olga Vitek, <sup>8</sup>Reudi Aebersold, <sup>18</sup>Leigh Anderson, <sup>19</sup>Robert Bethem, <sup>20</sup>Josip Blonder, <sup>20</sup>Emily Boja, <sup>21</sup>Julianne Botelho, <sup>13</sup>Michael Boyne; <sup>9</sup>Ralph A. Bradshaw, <sup>9</sup>Alma L. Burlingame; <sup>22</sup>Daniel Chan; <sup>1</sup>Hasmik Keshishian, <sup>1</sup>Eric Kuhn, <sup>20</sup>Christopher Kinsinger, <sup>20</sup>Jerry Lee, <sup>23</sup>Sang-Won Lee, <sup>5</sup>Robert Moritz, <sup>9</sup>Juan Osés-Prieto, <sup>24</sup>Nader Rifai, <sup>25</sup>James Ritchie, <sup>20</sup>Henry Rodriguez, <sup>26</sup>Pothur R. Srinivas, <sup>27</sup>R. Reid Townsend, <sup>22</sup>Jennifer Van Eyk, <sup>28</sup>Gordon Whiteley, <sup>9</sup>Arun Wiita and <sup>29</sup>Susan Weintraub

Source: Carr, SA, et. al. Targeted Peptide Measurements in Biology and Medicine: Best Practices for Mass Spectrometry-based Assay Development Using a Fit-for-Purpose Approach. (2014) *Mol Cell Proteomics*. 13(3):907-917.

# Alignment of Workshop Guidelines with CPTAC Assay Portal (<https://assays.cancer.gov>)

**Coming Soon!**

National Cancer Institute  
at the National Institutes of Health | [www.cancer.gov](http://www.cancer.gov)

OFFICE OF CANCER CLINICAL PROTEOMICS RESEARCH Assay Portal

Available Assays About CPTAC Home

Assay Portal

Search the Assay Database by:

KEGG Pathways  
Select

Data Source: KEGG

Find assays to proteins encoded in a specific chromosomal region

Chromosome Number  
Include All

Chromosomal Location  
Start Stop

Data Source: CPTAC Assay Database

Show / hide columns Download CSV

Showing 1 to 50 of 456 entries

Proteins and peptides for which assays are available

Submitting Laboratory

Modification

Assay Type

Matrix

CPTAC ID

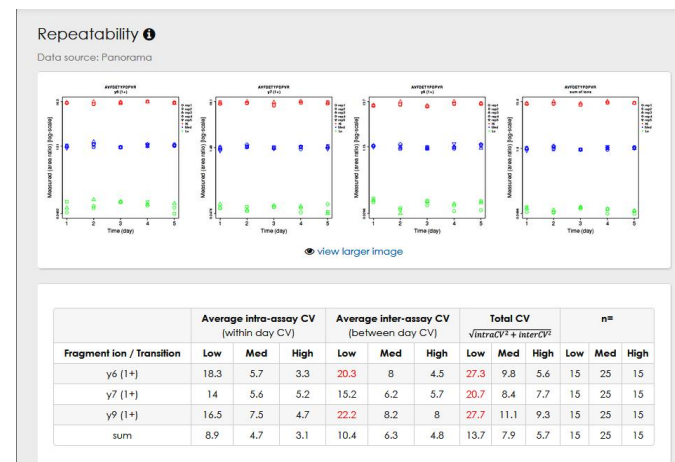
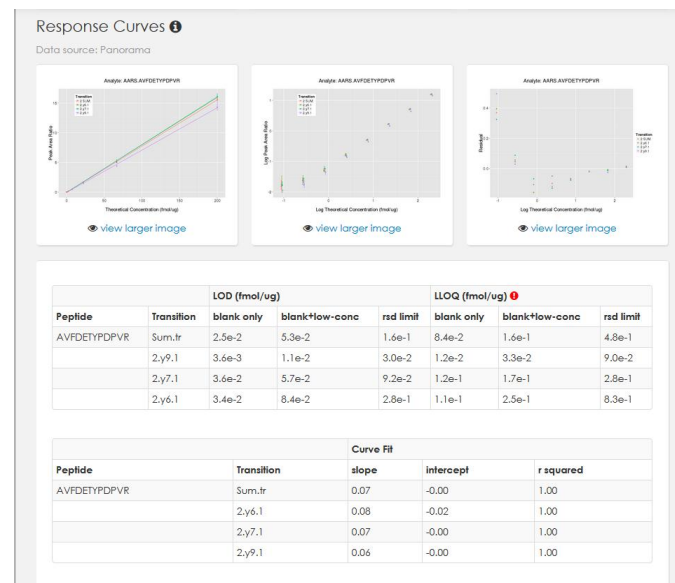
⊖ AARS - UniProt Accession ID: P49588

|                   |                                        |            |            |                       |         |
|-------------------|----------------------------------------|------------|------------|-----------------------|---------|
| AVFDEYDPVVR       | Fred Hutchinson Cancer Research Center | unmodified | direct MRM | cell line lysate pool | CPTAC-1 |
| TITVALADGGRPDNTGR | Fred Hutchinson Cancer Research Center | unmodified | direct MRM | cell line lysate pool | CPTAC-2 |

⊖ ABAT - UniProt Accession ID: P80404

|                |                                        |            |            |                       |         |
|----------------|----------------------------------------|------------|------------|-----------------------|---------|
| GTFCSDTPDDSIIR | Fred Hutchinson Cancer Research Center | unmodified | direct MRM | cell line lysate pool | CPTAC-4 |
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⊖ ABGDS - UniProt Accession ID: P28288

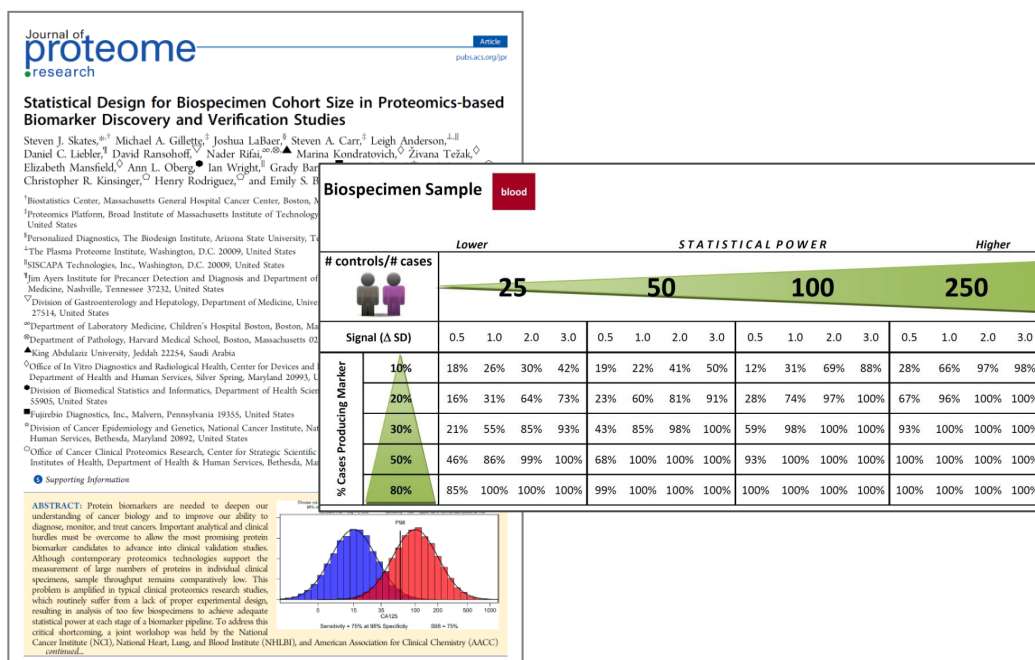


**Defining qualification criteria for different tiers of targeted assays**

# Experimental and Statistical Study Design Considerations for Discovery and Verification Stages



- **Workshop** – building consensus towards statistically correct biospecimen cohort size *when using multiplex proteomic technologies*
- **Outcome:** a peer-reviewed paper (modeling based on CA-125 for cohort size calculation; foundation for tumor analysis)



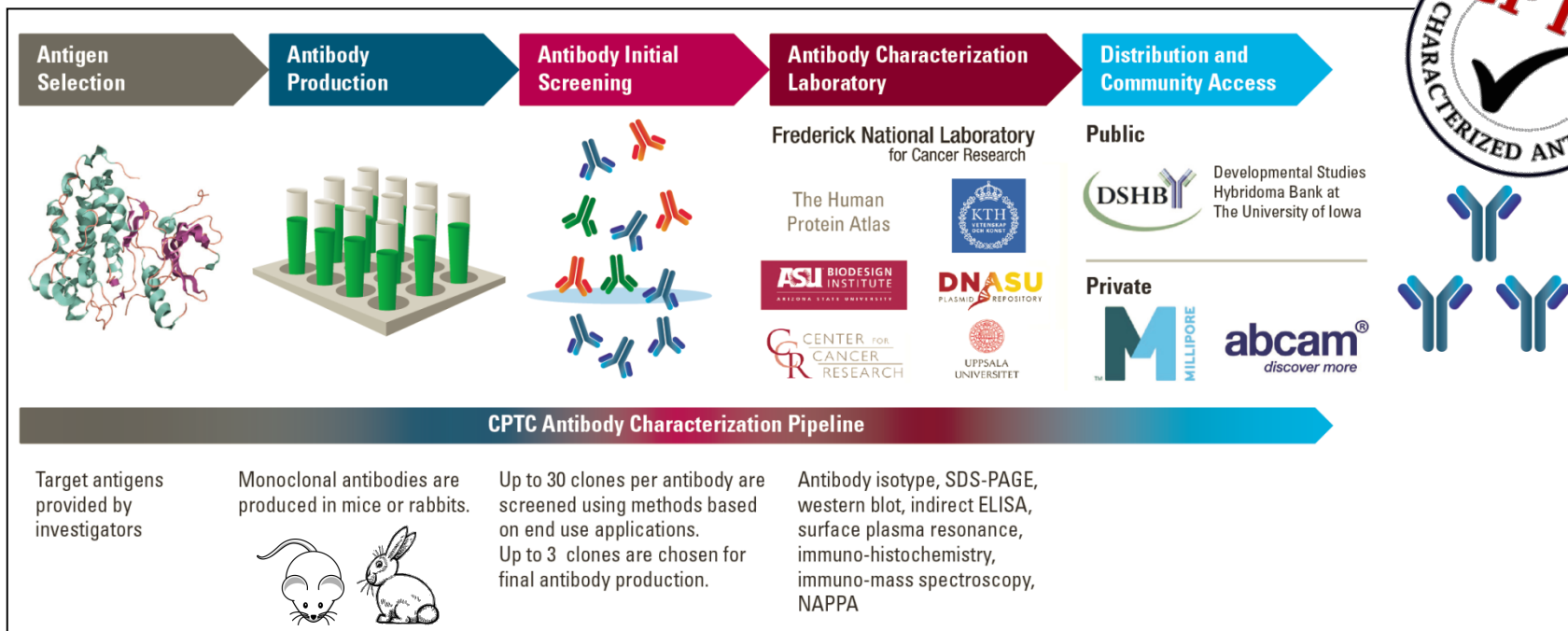
Source: Skates, S, Boja, ES, et. al. Statistical Design for Biospecimen Cohort Size in Proteomics-based Biomarker Discovery and Verification Studies. (2013) *J Proteome Res.* 12(12):5383-5394.



# Community Reagents: Monoclonal Antibodies (Well-characterized / Standardized)

## Antibody Characterization Program

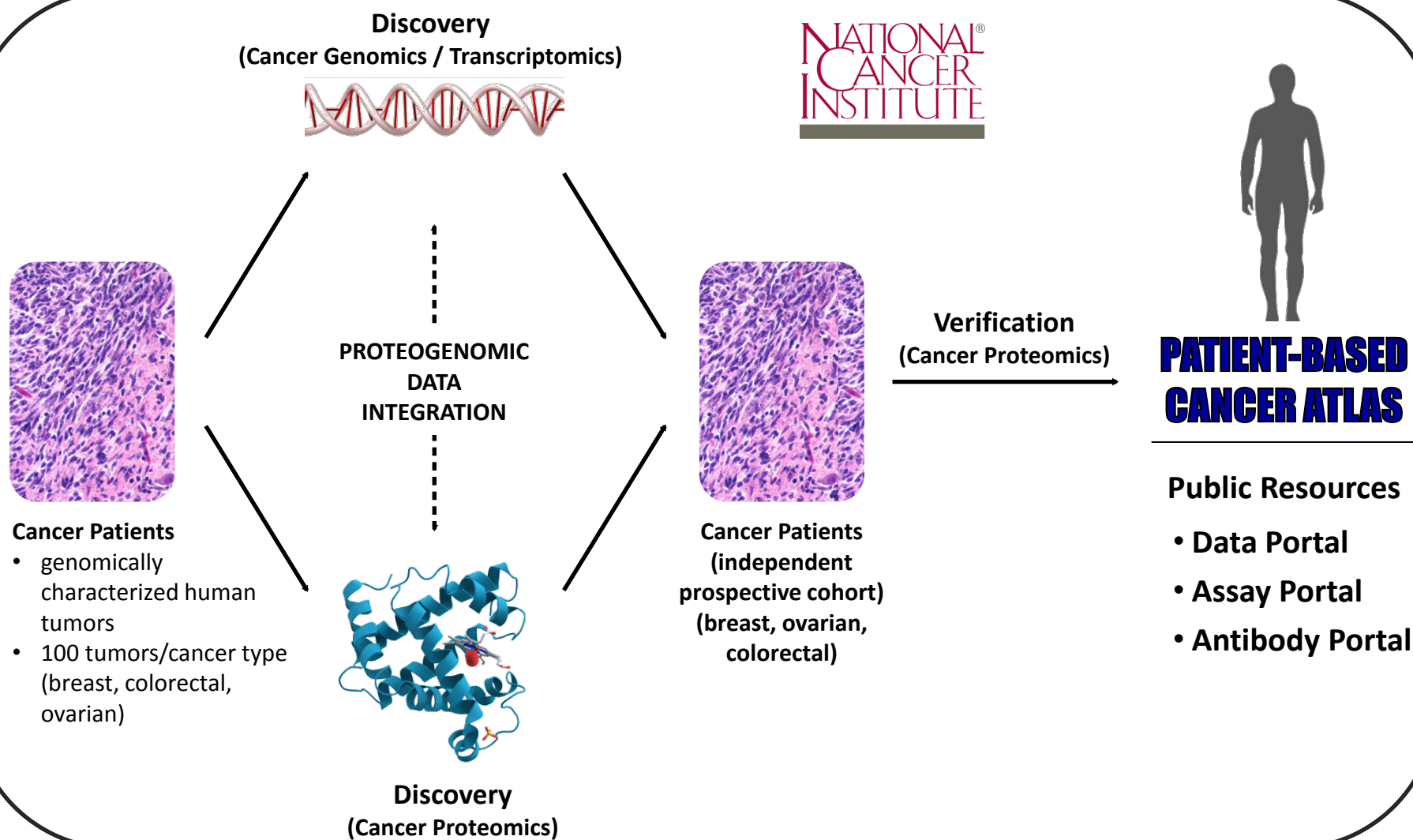
(<http://antibodies.cancer.gov>)



- Current Inventory: **295 validated monoclonal antibodies (126 full-length protein and peptide antigens)**



# Clinical Proteomic Tumor Analysis Consortium: Proteogenomics for Understanding Cancer Biology

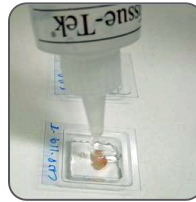


# Year 1: Understanding TCGA Pre-analytical Variables

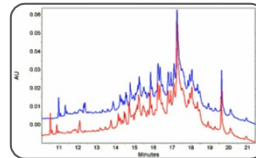


## Due Diligence Studies

1. Impact of Optimal Cutting Temperature compound on proteomic analysis?



2. Accounting for analytical/instrument drift within and among labs?



3. Impact of cold ischemia duration on proteome analysis?



## Outcome

- ✓ Developed OCT removal protocol (no negative impact)
- ✓ Developed CompRef (WHIM breast xenograft) standard – allows analytical benchmarking (every 5 TCGA sample runs)
- ✓ Developed consensus tumor collection SOPs for verification set – minimize ischemic effect on proteomic (phospho) analyses



*What has NCI-CPTAC done with FDA/AACC to bridge the gap?*

- Standardization of MS-based proteomic assays
  - Discovery (reference materials, MS QC software)
  - Verification: publicly available 510(k) mock documents using targeted immuno-MRM-MS assays
  - Experimental design considerations (power calculation, bias)
  - Standardization of monoclonal affinity reagents characterization generated for proteomics research
- Standardization of common data analysis pipeline (NIST): beneficial for reporting of multi-institutional TCGA proteomic results to the public (PSMs)

# CPTAC Public Resources (Standardization)

LIVE

<https://cptac-data-portal.georgetown.edu/cptacPublic/>



CPTAC Data Portal

11,419 files (2.2 TB)

LIVE

<https://antibodies.cancer.gov>



NCI Antibody Portal

295 mAbs



COMING SOON

<https://assays.cancer.gov>



CPTAC Assay Portal

542 assays



Download Data About the Data Help CPTAC Home Antibody Portal

CPTAC, TCGA Cancer Proteome Study of Colorectal Tissue  
Embargo Release Date: December 04, 2014

The goal of the CPTAC, TCGA Cancer Proteome Study of Colorectal Tissue is to analyze the proteomes of TCGA tumor samples that have been comprehensively characterized by molecular methods (Cancer Genome Atlas Network, Nature 2012)

Data Sets

Download

| Name                                                    | Size  |
|---------------------------------------------------------|-------|
| CPTAC TCGA Colorectal Cancer Proteome and Clinical Data | 839KB |
| TCGA-AA-3807-G1A-22 Proteome_VU_20121019                | 8GB   |
| TCGA-AA-3808-G1A-22 Proteome_VU_20121205                | 9GB   |
| TCGA-AA-3810-G1A-22 Proteome_VU_20121029                | 8GB   |
| TCGA-AA-3518-G1A-11 Proteome_VU_20120915                | 8GB   |
| TCGA-AA-3525-G1A-12 Proteome_VU_20121209                | 8GB   |
| TCGA-AA-3526-G1A-11 Proteome_VU_20130106                | 8GB   |
| TCGA-AA-3529-G1A-12 Proteome_VU_20121203                | 9GB   |
| TCGA-AA-3531-G1A-22 Proteome_VU_20130101                | 8GB   |
| TCGA-AA-3534-G1A-22 Proteome_VU_20130208                | 7GB   |
| TCGA-AA-3532-G1A-22 Proteome_VU_20130125                | 8GB   |
| TCGA-AA-3554-G1A-22 Proteome_VU_20121122                | 8GB   |
| TCGA-AA-3807-G1A-22 Proteome_VU_20121019                | 8GB   |
| TCGA-AA-3808-G1A-22 Proteome_VU_20121205                | 9GB   |

Available Antibodies About CPTAC Home

Antibody Portal

Browse By Antibodies

Antigen Recognition Show 25 entries Showing 1 to 25 of 231 entries Search:

recombinant full-length

| Antibody Images Types                             | Antibodies       | Antigens                                        | Purchase                 |
|---------------------------------------------------|------------------|-------------------------------------------------|--------------------------|
| <input type="checkbox"/> SPR                      | CPTC-AKR1B1-1    | Aldo-keto Reductase Family 1 Member B1          | <input type="checkbox"/> |
| <input type="checkbox"/> 3HC NCI80                | CPTC-AKR1B1-2    | Aldo-keto Reductase Family 1 Member B1          | <input type="checkbox"/> |
| <input type="checkbox"/> NMRPA                    | CPTC-AKR1B1-3    | Aldo-keto Reductase Family 1 Member B1          | <input type="checkbox"/> |
| <input type="checkbox"/> Immuno-MS                | CPTC-AKR1C1-1    | Aldo-keto Reductase Family 1 Member C1          | <input type="checkbox"/> |
| <input type="checkbox"/> Western Blot             | CPTC-AKR1C1-2    | Aldo-keto Reductase Family 1 Member C1          | <input type="checkbox"/> |
| <input type="checkbox"/> Indirect ELISA           | CPTC-AKR1C2-1    | Aldo-keto reductase family 1 member C2          | <input type="checkbox"/> |
| <input type="checkbox"/> NCI 60 Protein Array     | CPTC-AKR1C2-2    | Aldo-keto reductase family 1 member C2          | <input type="checkbox"/> |
| <input type="checkbox"/> 3HC HPA                  | CPTC-AKR1C2-3    | Aldo-keto reductase family 1 member C2          | <input type="checkbox"/> |
| <input type="checkbox"/> Cell Lysate Western Blot | CPTC-ABGA1-1     | Annexin A1 (Annexin I)                          | <input type="checkbox"/> |
| <input type="checkbox"/> Cross Reactivity Data    | CPTC-ABGA1-2     | Annexin A1 (Annexin I)                          | <input type="checkbox"/> |
| <input type="checkbox"/> 3HC Tissue               | CPTC-ABGA1-3     | Annexin A1 (Annexin I)                          | <input type="checkbox"/> |
|                                                   | CPTC-APDX1-1     | APDX1 Nuclease 1                                | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L1-1    | BCL2 like 1                                     | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L1-2    | BCL2 like 1                                     | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L1-3    | BCL2 like 1                                     | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L2-1    | BCL2 like 2                                     | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L2-2    | BCL2 like 2                                     | <input type="checkbox"/> |
|                                                   | CPTC-BCL2L2-3    | BCL2 like 2                                     | <input type="checkbox"/> |
|                                                   | CPTC-CAB-1       | Carbonic anhydrase VIII                         | <input type="checkbox"/> |
|                                                   | CPTC-CAB-2       | Carbonic anhydrase VIII                         | <input type="checkbox"/> |
|                                                   | CPTC-Calcyclin-1 | Calcyclin (Protein Receptor Associated Protein) | <input type="checkbox"/> |
|                                                   | CPTC-Calcyclin-2 | Calcyclin (Protein Receptor Associated Protein) | <input type="checkbox"/> |
|                                                   | CPTC-Calcyclin-3 | Calcyclin (Protein Receptor Associated Protein) | <input type="checkbox"/> |
|                                                   | CPTC-CDCA3-1     | Cell division cycle 34 homolog (S. cerevisiae)  | <input type="checkbox"/> |

Antibody isotypes

☐ IgG1

☐ IgG2a

☐ IgG2b

☐ IgG3

Monoclonal Source

☐ Mouse

External Links

☐ Human Protein Atlas

☐ QDAB

Available Assays About CPTAC Home

Assay Portal

Search the Assay Database by:  Show / hide columns

Clear All Filters

KEGG Pathways

Data Source: KEGG

Find assays to quantify proteins that interact with the following protein(s):

Data Source: BioGRID

Find assays that work

| Proteins and peptides for which assays are available | Submitting Laboratory                                                 | Modification | Assay Type | Matrix                |
|------------------------------------------------------|-----------------------------------------------------------------------|--------------|------------|-----------------------|
| <b>AARS - UniProt Accession ID: P49588</b>           |                                                                       |              |            |                       |
| AVFDEPDPVR                                           | Fred Hutchinson Cancer Research Center                                | unmodified   | direct MRM | cell line lysate pool |
| ITVALADGQFQNTGR                                      | Fred Hutchinson Cancer Research Center                                | unmodified   | direct MRM | cell line lysate pool |
| <b>ABAT - UniProt Accession ID: P80404</b>           |                                                                       |              |            |                       |
| ALTGLDLQAR                                           | Fred Hutchinson Cancer Research Center                                | unmodified   | direct MRM | cell line lysate pool |
| GTFCSDFPDQSR                                         | Fred Hutchinson Cancer Research Center                                | unmodified   | direct MRM | cell line lysate pool |
| <b>ABCD5 - UniProt Accession ID: P28288</b>          |                                                                       |              |            |                       |
| VGLGLWPLFGGR                                         | Seoul National University / Korea Institute of Science and Technology | unmodified   | direct MRM | cell line lysate pool |
| VGLGLWPLFGGR                                         | Fred Hutchinson Cancer Research Center                                | unmodified   | direct MRM | cell line lysate pool |

Data release schedule:

CrCa – completed

BrCa – completed

OvCa – 7/2014





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- Karin Rodland
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- Matt Mutch
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## Data Generation WG

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